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13. ABSTRACT *(Maximum 200*

There is a region on human chromosome 11 at p15.5 that has been associated with the breast cancer phenotype. These results were obtained by loss of heterozygosity (LOH) studies in breast tumors. Metastasis and poor outcome are the cancer phenotypes associated with 11p15.5. Since breast cancer patients usually do not die as a result of the primary tumor, but from the progression to a metastatic phenotype, we have embarked on a study to identify the gene(s) responsible on 11p15.5. We have constructed a detailed physical map across the region, participated in sequencing the region, generated a transcript map across the region and have begun to access the genes in this region as candidates for tumor suppressor genes. The candidate region has been reduced to 400 kb and contains about 4-6 potential genes. We have cloned the region and the molecular characterization has begun. Interestingly, the 11p15.5 region has been shown to be located in an imprinted domain. We have evidence to suggest preferential 11p15.5 maternal loss in breast tumors suggesting that breast cancer in this region has an imprinting component. We have cloned the homologous imprinted region from the mouse and are testing for imprinted expression in fetal and adult tissues including mammary gland.

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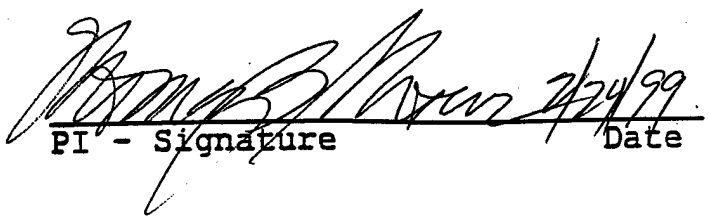

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IDENTIFYING AND ISOLATING BREAST CANCER-ASSOCIATED GENES ON CHROMOSOME 11

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STATEMENT OF WORK

Task 1. Refining and narrowing the human chromosome 11 region associated with the breast cancer phenotype between genes CALCA and HBB. This project has been completed and the region narrowed further to 400 kb by loss of heterozygosity (LOH) studies between D11S4088 and D11S1318 in the 11p15.5 region.

Task 2. Complete a contig across this region. This project has been completed and the smallest candidate region has been sequenced.

Task 3. Identify candidate genes in the implicated region. This project is near completion. Eighteen genes have been identified and mapped to the smallest candidate region. Greater than 90% of the region has been analyzed and a transcript map generated.

Task 4. Analyze candidate genes. This project is well underway and the results are indicating that additional chromosomal phenomenon besides tumor suppressor genes are involved.

Task 5. Examine candidate tumor suppressor genes. Methodology has been optimized and several genes have been investigated and characterized.

Task 6. Develop and characterize those gene markers that identify tumor suppressor genes in breast cancer. This task is underway.

Task 7. Investigate the involvement of imprinting in breast cancer and the identification of an imprinting center in the smallest candidate region.

PROGRESS ACHIEVED

Task 1. Refine and narrow the region between genes CALCA and HBB on human chromosome 11p15.5. This task has been completed and has been presented on previous year's progress reports (see Task 2 for a publication).

Task 2. Completed a physical (DNA) map across the 11p15.5 candidate region and analyzed it for verification and integrity. This task has been completed and presented last year and published (Reid et al., Genomics 43: 368-375, 1997).

We have successfully covered in PAC (P1 artificial chromosome) clones the 11p15.5 multiple tumor association region and closed the contigs, (overlapping cloned pieces of DNA)

covering ~700 kb between the DNA markers D11S517 and H19. This map allowed the precise location of all the known genes in the region including CARS, NAP2, p57KIP2, KVLQT1,TAPA1, ASCL2, TH, INS, IGF2, H19 and L23MRP.

Task 3. Isolation of novel genes by direct cDNA selection and by DNA sequencing.

In order to identify potential tumor suppressor genes and/or loci important in the etiology of the breast cancer phenotype, we have developed a comprehensive transcript or gene map through the large-insert bacterial clone (BAC) contig between D11S601 and D11S1318. This was accomplished by (1) fine mapping of genes previously mapped to 11p15.5, (2) direct cDNA selection using large-insert clones, and (3) large scale sequencing of PACs. We consider this task virtually completed. A detailed description was presented last year. We have published the transcript map of the candidate region (Crider-Miller et al., Genomics 46: 355-363, 1997; See Figure 1).

Task 4. Analyze candidate genes in the region of interest.

Genes are being analyzed in the candidate region on chromosome 11 as described in detail in last years report. Some of this work has been published, other work is in manuscript (Cooper et al., Genomics 49: 38-51, 1998; Smilinich et al., Proc. Natl. Acad. Sci., USA, submitted).

Task 5. Examine candidate genes in breast cancer patients.

We have examined two genes thus far by SSCP. They are NAP (we first isolated this gene, Rodriguez et al., Genomics 44: 253-265, 1997) and p57. No mutations have been found. Characterization of several genes in the candidate region is underway.

Task 6. Develop and characterize those gene markers that identify tumor suppressor genes in breast cancer.

We have narrowed the candidate region by LOH (loss of heterozygosity) studies of breast tumor (See Figure 2; higher resolution than last year). This region has been reduced to about 400 kb located between markers D11S4088 and D11S1318 in the p15.5 region of chromosome 11 (methodology is described in last year's report; See updated Figure 3). The region contains 4-6 transcripts, one of which may act as a tumor suppressor gene. Studies are underway to isolate and characterize these genes. This localization places the chromosomal region associated with breast cancer directly in an imprinting region as we have described (Cooper et al., Genomics 49: 38-51, 1998). Because of this new development, this project now has a Task 7 designed to investigate the involvement of imprinting in the breast cancer phenotype.

Task 7. Investigate the involvement of imprinting in breast cancer and possible identification of an imprinting center in the candidate region.

Our evidence demonstrates now that there is a component of breast cancer that is located on chromosome 11 in the 11p15.5 region, between markers D11S4088 and D11S1318, that is, in fact, imprinted. The LOH evidence suggests a preferential maternal loss in tumors which implies that a 11p.15.5 breast cancer tumor suppressor gene is imprinted. Figure 4 presents evidence for this finding. Wilms' tumor, on the left of the figure as control, is know to show preferential loss of the maternal chromosome as shown in the first three lanes for the maternal band and not the paternal band. These bands are alleles at the same locus in 11p15.5. Breast cancer is demonstrated on the right. The first four lanes are from breast cancer and the last three lanes are from normal breast tissue. The upper band (mat) represents a marker from the candidate region on the maternal chromosome. In normal tissue, both mat and pat bands (alleles) show equal intensity. In the breast tumor, there is a consistent loss of intensity in the upper (mat) band showing the maternal band to be lost consistently. The activity remaining in the upper band is considered to be residual activity from normal tissue in the sample. We are able to isolate Wilms' tumor tissue free of normal (left side) which is not the case for breast tumor (right side). These results imply the preferential loss of maternal genes in breast tumors. Normally, we would expect to see a random loss of bands (alleles) in a non-imprinted region and we have evidence to show such a random loss in non-11p15.5 loci in tumors. The results in Figure 4 are consistent with the other genes that show maternal expression in the 11p15.5 chromosomal region. Our results imply then, a maternally expressed tumor suppressor gene associated with breast cancer.

We have turned to the mouse to test this imprinted region further in mammary glands and under experimental conditions. A PAC contig was generated across the homologous chromosomal candidate region on mouse distal chromosome 7. We have published this map (Day et al., 1999). This homologous mouse region was shown to be imprinted previously and we have confirmed this. Having this region cloned in the mouse gives us the reagents for sequencing, mutagenic studies and analyzing these genes in the mammary gland and studying imprinting and its involvement in breast cancer.

SUMMARY FOR THIS YEAR

1) Narrowed the breast cancer 11p15.5 region from 3000 kb to less than 400 kb (8-fold reduction) by LOH.

2) This region contains 4-6 transcripts (potential genes), one of which may act as a tumor suppressor gene. This region has been cloned.

3) The 11p15.5 breast cancer LOH region is located within an imprinted "domain". For the first time, the preferential maternal loss in tumors suggest that a breast cancer tumor suppressor gene is imprinted.

4) We have isolated the homologous region in the mouse and are testing for imprinted expression in fetal and adult tissue including mammary gland.

PUBLICATIONS

Cooper, P.R., Smilinich, N.J., Day, C.D., Nowak, N.J., Reid, L.H., Pearsall, R.S., Reece, M., Prawitt, D., Landers, J., Housman, D.E., Winterpacht, A., Zabel, B.U., Pelletier, J., Weissman, B.E., Shows, T.B. and Higgins, M.J. 1998. Divergently transcribed overlapping genes expressed in liver and kidney and located in the 11p15.5 imprinted domain. Genomics 49: 38-51.

Crider-Miller, S.J., Reid, L.H., Higgins, M.J., Nowak, N.J., Shows, T.B., Futreal, P.A. and Weissman, B.E. 1997. Novel transcript sequences within the BWS/WT2 region in 11p15.5: Tissue-specific expression correlates with cancer type. Genomics 46: 355-363.

Day, C.D., Smilinich, N.J., Fitzpatrick, G.V., de Jong, P.J., Shows, T.B. and Higgins, M.J. (1999) The imprinted domain in distal chromosome 7: Reagents for mutagenesis and sequencing. Mammalian Genome 10: 182-185.

Reid, L.H., Davies, C., Cooper, P.R., Crider-Miller, S.J., Sait, S.N.J., Nowak, N.J., Evans, G., Stanbridge, E.J., de Jong, P., Shows, T.B., Weissman, B.E. and Higgins, M.J. 1997. A 1-Mb physical map and PAC contig of the imprinted domain in 11p15.5 that contains the TAPA1 and the BWSCR1/WT2 region. Genomics 43: 366-375.

Rodriguez, P., Munroe, D., Prawitt, D., Chu, L.L., Bric, E., Kim, J., Reid, L.H., Davis, C., Nakagama, H., Loebbert, R., Winterpacht, A., Petruzzi, M.J., Higgins, M.J., Nowak, N., Evans, G., Shows, T., Weissman, B.E., Zabel, B., Housman, D.E. and Pelletier, J. 1997. Functional characterization of human nucleosome assembly protein-2 (NAP1L4) suggests a role as a histone chaperone. Genomics 44: 253-265.

Smilinich, N.J., Day, C.D., Fitzpatrick, G.V., Caldwell, G.M., Lossie, A.C., Cooper, P.R., Smallwood, A.C., Joyce, J.A., Schofield, P.N., Reik, W., Nicholls, R.D., Driscoll, D.J., Maher, E.R., Shows, T.B. and Higgins, M.J. 1999. A maternally methylated CpG-island in KvLQT1 is associated with an antisense paternal transcript and loss of imprinting. Proc. Natl. Acad. Sci., USA, submitted.

ABSTRACTS

Cooper, P.R., Reid, L.H., Crider-Miller, S.J., Pelletier, J., Pearsall, R.S., Zabel, B.U., Weissman, B.E., Shows, T.B. and Higgins, M.J. 1998. Towards the molecular dissection of the imprinted domains in human chromosome band 11p15.5 and mouse distal chromosome 7. 18th Annual Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada.

Higgins, M.J., Cooper, P.R., Nowak, N.J., Reid, L.H., Crider-Miller, S.J., Davies, C., Gabriel, J.M., Nicholls, R.D., deJong, P.J., Evans, G., Weissman, B.E. and Shows, T.B. 1997. Loss of imprinting at IGF2 and a novel CpG-island in a BWS fetus with an inversion chromosome 11. AACR Special Conference in Cancer Research, December12-16, Las Croabas, Puerto Rico.

Shows, T.B., Higgins, M.J. and Nowak, N.J. 1998. Identifying breast cancer-associated genes on human chromosome 11. AACR Annual Mtg., March 28-April 1, New Orleans, LA.

Shows, T.B., Higgins, M.J. and Nowak, N.J. 1997. Identifying and isolating breast cancer-associated genes on chromosome 11. Department of Defense, U.S. Army Breast Cancer Meeting, An Era of Hope, October 30-November 3, Washington, D.C.

Figure 1

Transcript Map of the Breast Cancer LOH Region

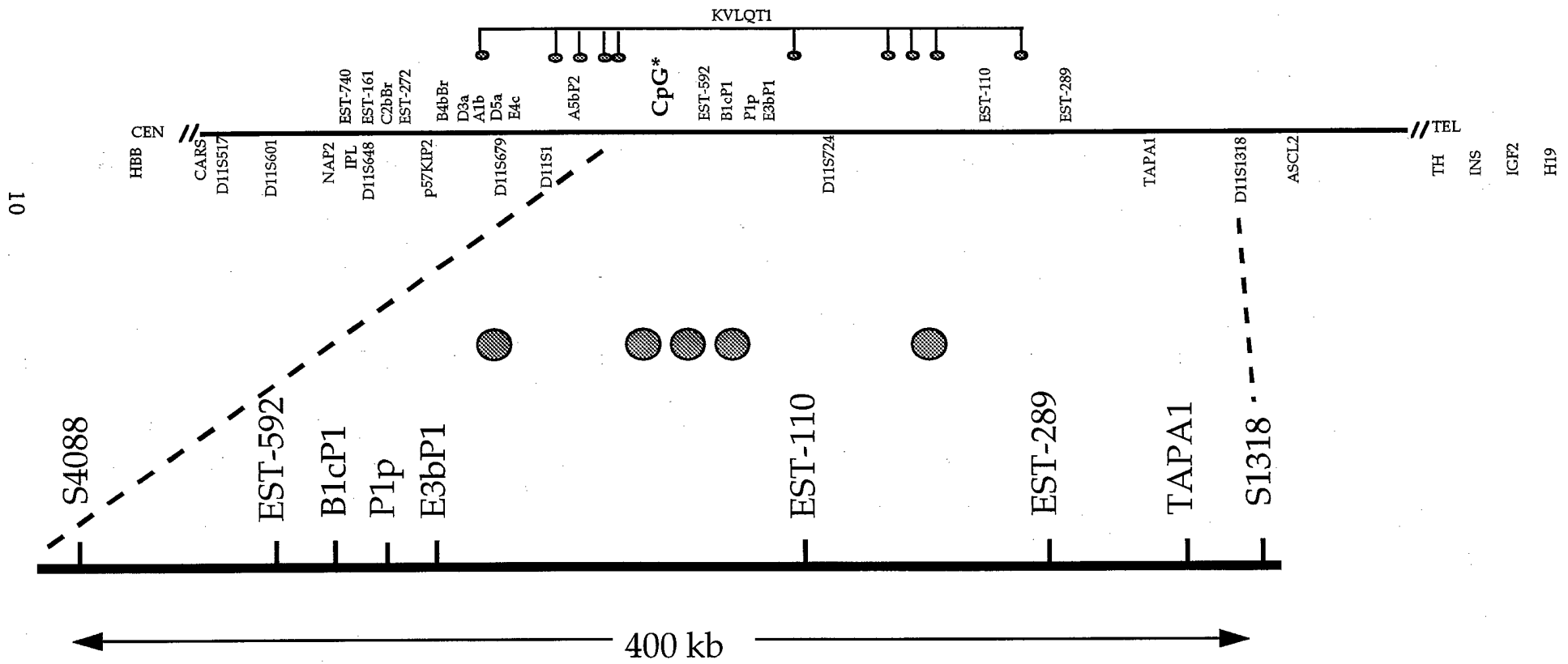


Figure 2

11p15.5 Breast Cancer LOH Region Narrowed to 400 kb between *D11S4088* and *D11S1318*

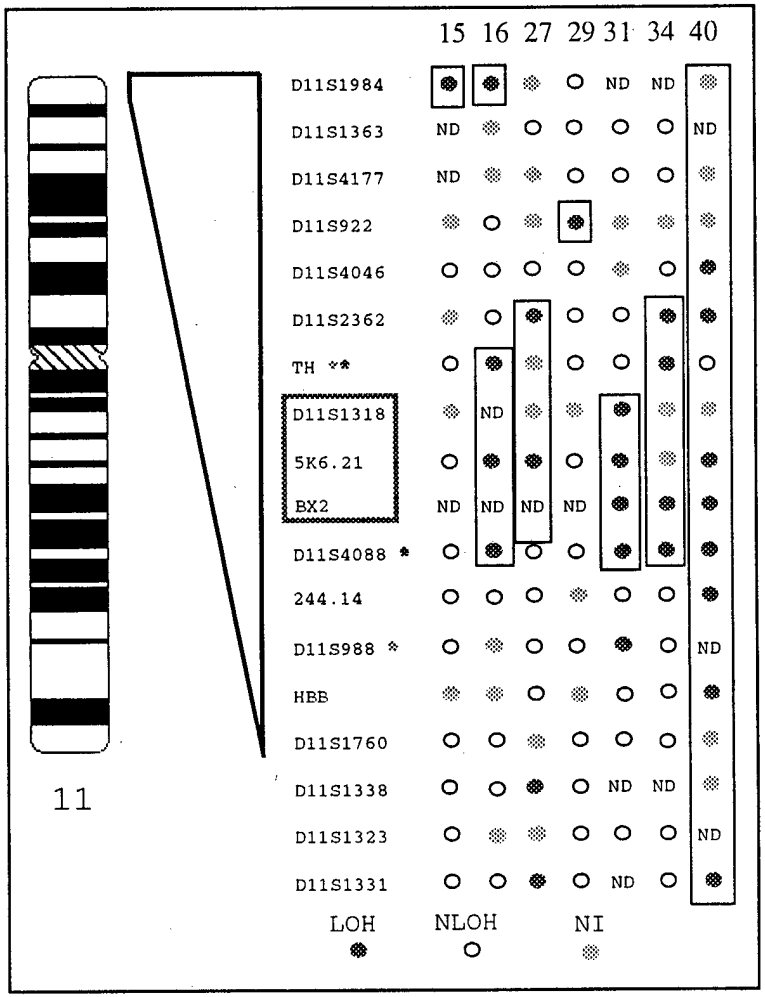


Figure 3

11p15.5 Breast Cancer LOH Region Narrowed to 400 kb between *D11S4088* and *D11S1318*

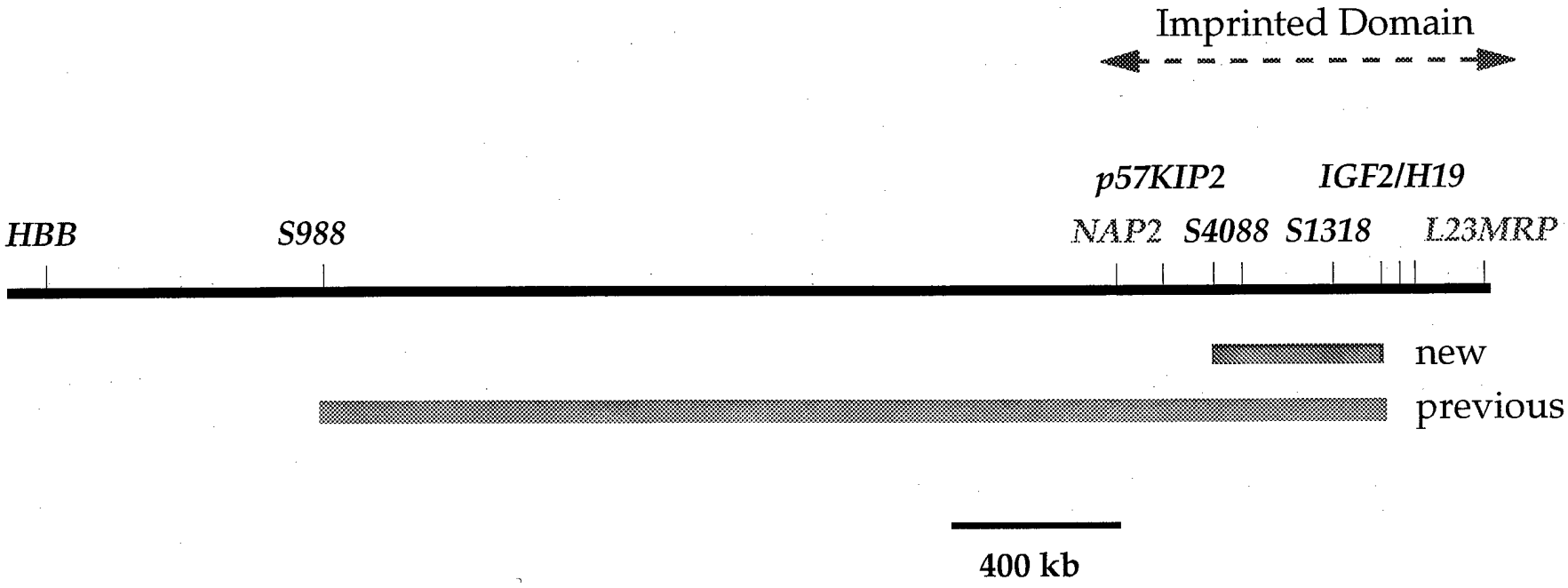
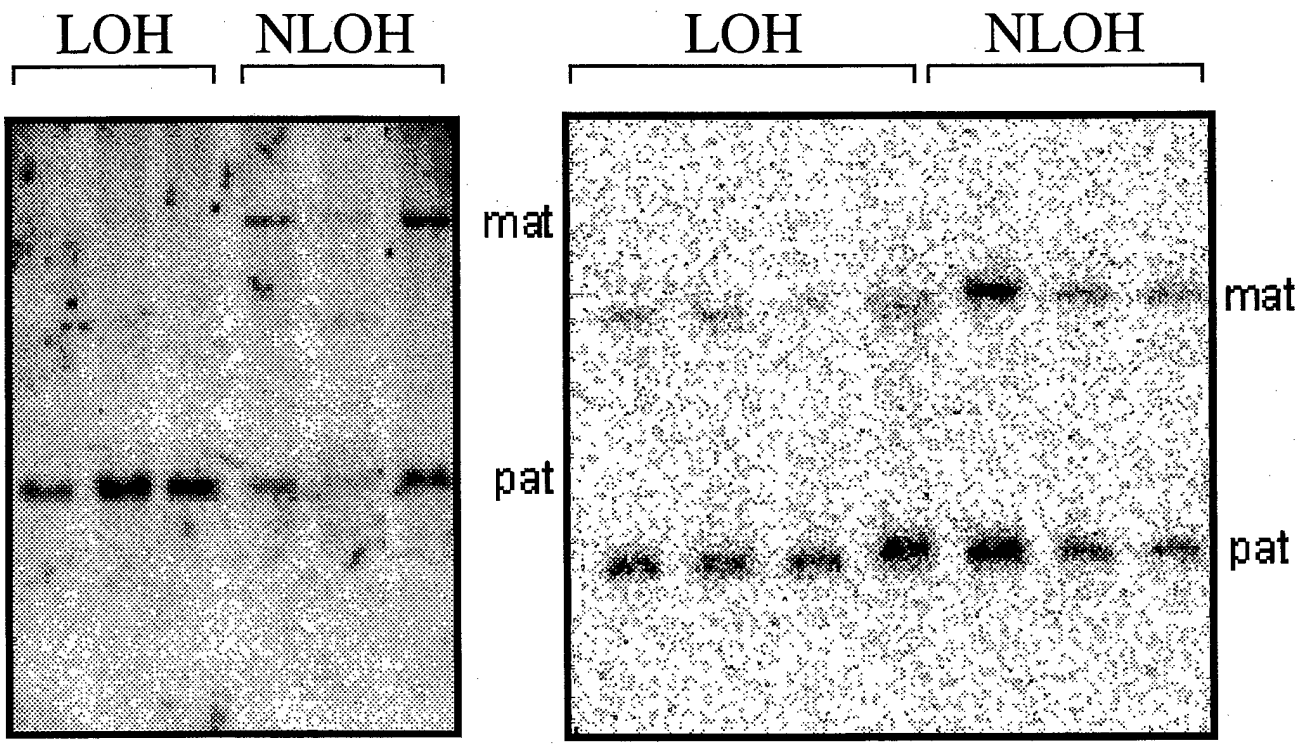


Figure 4

An Imprinted Breast Cancer Tumor Suppressor?



Wilms tumors

Breast tumors